Contributions to Entomology 75 (1) 2025, 15–19 | DOI 10.3897/contrib.entomol.75.e138001

CONTRIBUTIONS TO ENTOMOLOGY

SENCKENBERG

Research Article

New records of *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908 in northern Rhineland-Palatinate (Diptera, Psychodidae, Phlebotominae)

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https://zoobank.org/D50F77CF-5E75-403F-9C90-2903C19E3DA8

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Academic editor: Frank Menzel | Received 27 September 2024 | Accepted 9 January 2025 | Published 25 February 2025

Abstract

In the three consecutive years of 2022–2024, three female specimens of the phlebotomine sandfly *Phlebotomus mascittii* were trapped in two locations in the vicinity of Coblenz, western Germany. These records slightly expand the distribution limits of the species in the Federal State of Rhineland-Palatinate to the north, at latitudes 50°18'N and 50°15'N, respectively, and represent the second northernmost Palearctic sandfly locations so far.

Zusammenfassung

In den drei aufeinanderfolgenden Jahren 2022–2024 wurden drei weibliche Individuen der Sandmücke *Ph. mascittii* an zwei Standorten in der Umgebung von Koblenz, Westdeutschland, gefangen. Diese Funde verschieben die Verbreitungsgrenzen der Art im Bundesland Rheinland-Pfalz leicht nach Norden, bei 50°18'N bzw. 50°15'N, und repräsentieren damit die zweitnördlichsten paläarktischen Sandmücken-Funde überhaupt.

Key Words

Central Europe, Coblenz, Germany, horse stables, medical entomology, Phlebotominae, Psychodidae

Schlüsselwörte

Deutschland, Koblenz, Medizinische Entomologie, Mitteleuropa, Pferdeställe, Phlebotominae, Psychodidae

Introduction

One of the little-known taxa of hematophagous insects in Central Europe, including Germany, is the dipterous subfamily Phlebotominae of the family Psychodidae, also known as phlebotomine sandflies (in German "Sandmücken"). One reason for their neglect in Germany is the fact that they were only found here for the first time in 1999 (Naucke and Pesson 2000). Another reason might be their very small size (usually below 4 mm) coupled with nocturnal activity and a very hidden terrestrial lifecycle.

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However, in subtropical and tropical regions of the world, sandflies play a pivotal role as vectors of human and zoonotic pathogens, above all, of protozoan parasites of the genus *Leishmania* (Trypanosomatida: Trypanosomatidae) (Ready 2013; Maia et al. 2023). Infections with *Leishmania* spp. produce a variety of diseases in humans, some of which may have debilitating or even fatal outcomes. In the Mediterranean parts of Europe, mainly a visceral syndrome occurs, which is caused by *L. infantum* Nicolle, 1908, transmitted by sandfly species of the genus *Phlebotomus* (Ready 2013; Prudhomme et al. 2024). The natural reservoirs of the zoonotic parasite are mostly canids. As potentially infected stray dogs are often brought from the Mediterranean to Germany (Maia and Cardoso 2015), and as climate change is suspected to promote both the development and the spread of both the vectors and the parasites northwards, recent years have seen growing surveillance activities in Central Europe (Depaquit et al. 2005; Naucke et al. 2008, 2011; Kasbari et al. 2012; Poeppl et al. 2013; Oerther et al. 2020; Risueño et al. 2024).

Despite the quite recent first-ever record in Germany in the federal state of Baden-Württemberg in 1999 (Naucke and Pesson 2000), it can be argued that sandflies occurred here much earlier, as they were already reported from neighboring French Alsace-Vosges as early as 1950 (Schaffner 2023; Prudhomme et al. 2024). In the meantime, not only were new sandfly locations reported from southwest Germany between 49° and 50° northern latitudes (Oerther et al. 2020), but more northerly, isolated single records were reported from the German federal states of Rhineland-Palatinate at 50°19'N (Naucke et al. 2008) and Hesse at 50°35'N (Melaun et al. 2014). Those and most other records referred to *Ph. mascittii*, while four specimens of *Ph. perniciosus* Newstead, 1911, were found in 2001 (Naucke and Schmidt 2004). At least the latter is a proven vector of *L. infantum*, while *Ph. mascittii* is only suspected (Ready 2013; Obwaller et al. 2016). In order to extend the studies along the northern limits, we conducted occasional vector collections in the vicinity of Coblenz, western Germany, since 2022.

Materials and methods

The city of Coblenz is situated at the confluence of the large rivers Moselle and Rhine, in northern Rhineland-Palatinate, western Germany, at about 70 m altitude above sea level. It is surrounded by low mountain ranges (maximum altitude 881 m): the "Westerwald" to the Northeast, the "Taunus" to the Southeast, the "Hunsrück to the Southwest, and the "Eifel" to the West. The current study was conducted at or near horse stables with attached farms in four locations in the vicinity of Coblenz during the summer months of three consecutive years (Table 1).

All surveys were carried out between July and September during warm or hot summertime and without (or very little) rain, with daytime maximum temperatures of 25–30 °C and nighttime minimums between 15 and 20 °C.

Table 1. Details of trap locations and surveys.

Locality	Latitude/Longitude	Altitude in m a.s.l.	Study year
Mörz	50°15'N, 7°23'E	230	2022
Lahnstein	50°18'N, 7°37'E	180	2023, 2024
Coblenz (Güls)	50°20'N, 7°32'E	120	2024
Wolken	50°19'N, 7°27'E	260	2024

Geologically, the region is dominated by slate rock, which is deeply incised by the valleys of the Moselle and Rhine rivers, along which there are the renowned Riesling wineries. The rock base is often overlayed by glacial loess soil.

For the collections of vectors, several trap types were alternately utilized: commercially available yellow sticky card fungus gnats traps (in Mörz and Lahnstein only; approx. 50 cm^2 each card), BG-Pro sentinel-style odor-baited traps with BG-Lure/Mozzibait/Sweetscent lure and BG-CO_2 generator (Biogents, Regensburg, Germany), New Standard Miniature Incandescent light traps model 1012 (J.W. Hock Co., Gainesville, USA), CDC Mini light traps model 2836BQ and UV LED CDC traps model 2770 (Bio-Quip, Ranch Dominguez, USA), or own prototypes of color-LED light traps (with red, orange, blue, green, and white LEDs). All odor-baited and light traps were operated overnight for approximately 14–16 hours, whereby the light traps were suspended approx. 80 cm from the ground. Most traps were located outdoors under open roofs, some in the semi-open stables. The sticky card traps were installed over a period between 4 days and 2 months. In total, light traps and BG traps together were operated for 45 trapnights: light traps 27 trapnights, BG-Pro traps 16 trapnights.

Catches were freeze-killed (except sticky traps) and subsequently sorted under a dissection microscope. For species identification, extracted sandflies were submerged in 70% ethanol, and then the head and apical third of the abdomen containing the genital terminalia were carefully removed, cleared in 3% potassium hydroxide (Remel/Thermo Fisher Scientific, Lenexa, USA), and finally mounted in 54 °C pre-heated phenol-free Kaiser's glycerol gelatine (Sigma Aldrich/Merck KGaA, Darmstadt, Germany). Morphological determination was carried out following the identification keys of Theodor (1958) and Dantas-Torres et al. (2014). The specimens (mounted on slides/nucleic acid extraction tube#) are deposited in our departmental reference collections under the following codes: slide 120822-1f/#22-873; slide 140723-1f/#23-562; slide 220724-1f/#24-481.

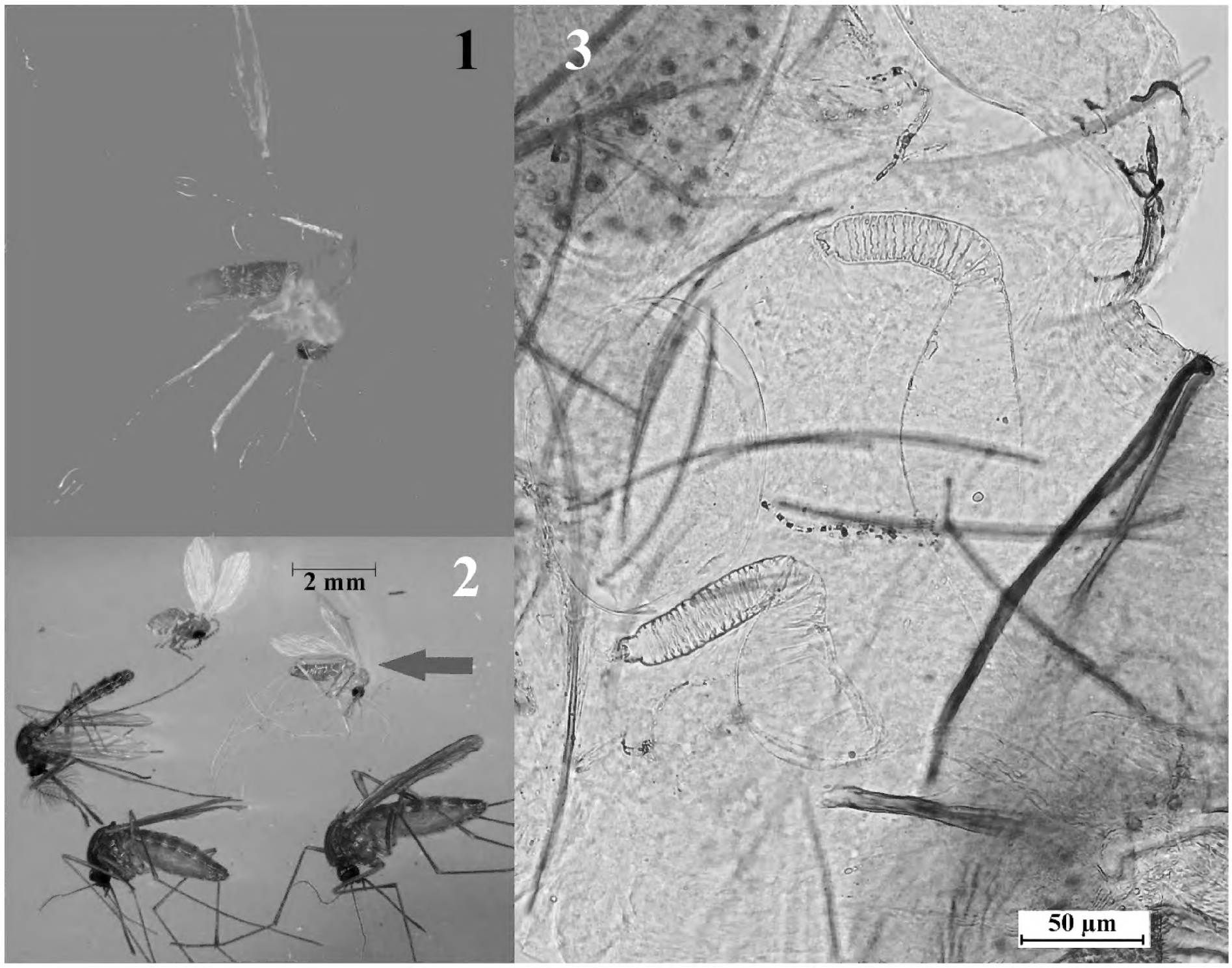
Results

Three female specimens of the phlebotomine sandfly *Phlebotomus mascittii* were trapped in two locations, Mörz and Lahnstein, between 2022 and 2024 (Table 2; Figs 1–3), whereby three different trap types yielded these specimens (Fig. 4): sticky card, BG-Pro+CO_2, and color-LED. Repeated trapping in the same year did not reveal further specimens at both sites, but the presence in Lahnstein could be confirmed one year later (Fig. 5). On the other hand, all surveys in the sites Coblenz and Wolken (all 2024) were negative.

Table 2. Capture sites, dates, trap types, and sand fly presence.

Locality	Date	Trap type	*Ph. mascittii* (positive / negative)
Mörz	23.7.–11.8.2022	Sticky	+
	23.–24.7.2022	CDC* & BG-Pro	-
	13.–14.8.2022	CDC* & BG-Pro	-
Lahnstein	11.–12.7.2023	BG-Pro, outdoor	+
		BG-Pro, indoor	-
		CDC* outdoor	-
	11.7.–11.9.2023	Sticky	-
	11.–12.9.2023	BG-Pro, CDC*, outdoor	-
	19.–20.7.2024	LED color, outdoor	+
		BG-Pro, LED color2, CDC Mini, all outdoor	-
	19.–23.7.2024	Sticky, outdoor	-
	22.–23.7.2024	BG-Pro, 2× LED color, CDC Mini, all outdoor	-
	7.–8.9.2024	BG-Pro, CDC-UV	-
Coblenz (Güls)	5.–6.8.2024	BG-Pro, CDC-UV+CO_2, LED color+CO_2, all outdoor	-
	12.–13.8.2024	BG-Pro, LED color+CO_2, all outdoor	-
	16.–17.8.2024	LED color +CO_2, LED color, all outdoor	-
	22.–23.8.2024	BG-Pro, LED color+lure, CDC Mini +lure, all outdoor	-
	6.–7.9.2024	BG-Pro, 2× LED color, all outdoor	-
Wolken	13.–14.8.2024	2× BG-Pro, LED color, outdoor; LED color+CO_2 indoor	-
	1.–2.9.2024	2× BG-Pro, LED color, CDC Mini, outdoor; LED color indoor	-

* any of the CDC-style light trap models.



Figures 1–3. Females of *Phlebotomus mascittii*. **1.** Female adult on a yellow sticky card trap collected in Mörz, 2022; **2.** Female adult (green arrow) caught with a BG-Pro trap in Lahnstein, 2023, together with a psychodid moth fly (top left) and three *Culex* mosquitoes (bottom); **3.** Spermathecae of a female from Lahnstein collected with a color-LED prototype trap in 2024.

Both positive and negative sites were not only characterized by being horse stables, but there were also other farm animals and pets present (donkeys, goats, pigs, sheep, dogs, cats), as well as many visitors (horse enthusiasts).

Discussion

Fig. 6 puts our new findings in context with previously known locations in the neighboring federal and national states of Hesse, Baden-Württemberg, Luxembourg, Belgium, and France (Depaquit et al. 2005; Naucke et al. 2008; Melaun et al. 2014; Oerther et al. 2020; Risueño et al. 2024). Geographically, the northern limit of *Ph. mascittii* is now demarcated by the Moselle and Lahn river valleys between the border triangle of France, Luxembourg, and Germany, and the isolated site in Giessen. Still, Giessen continues to be the northernmost Palearctic or even worldwide site (Melaun et al. 2014), but our new findings narrow the gap to the other German localities. However, the Cochem report of Naucke et al. (2008) contained a typing error (T. Naucke, pers. comm.). The given latitude of

Figures 4, 5. Collection points and trap positions in Lahnstein. **4.** Prototype of a color-LED trap (with a green bag) and a yellow sticky card trap underneath in 2024; **5.** The positive site with a BG-Pro trap used in 2023 and 2024 (yellow arrow).

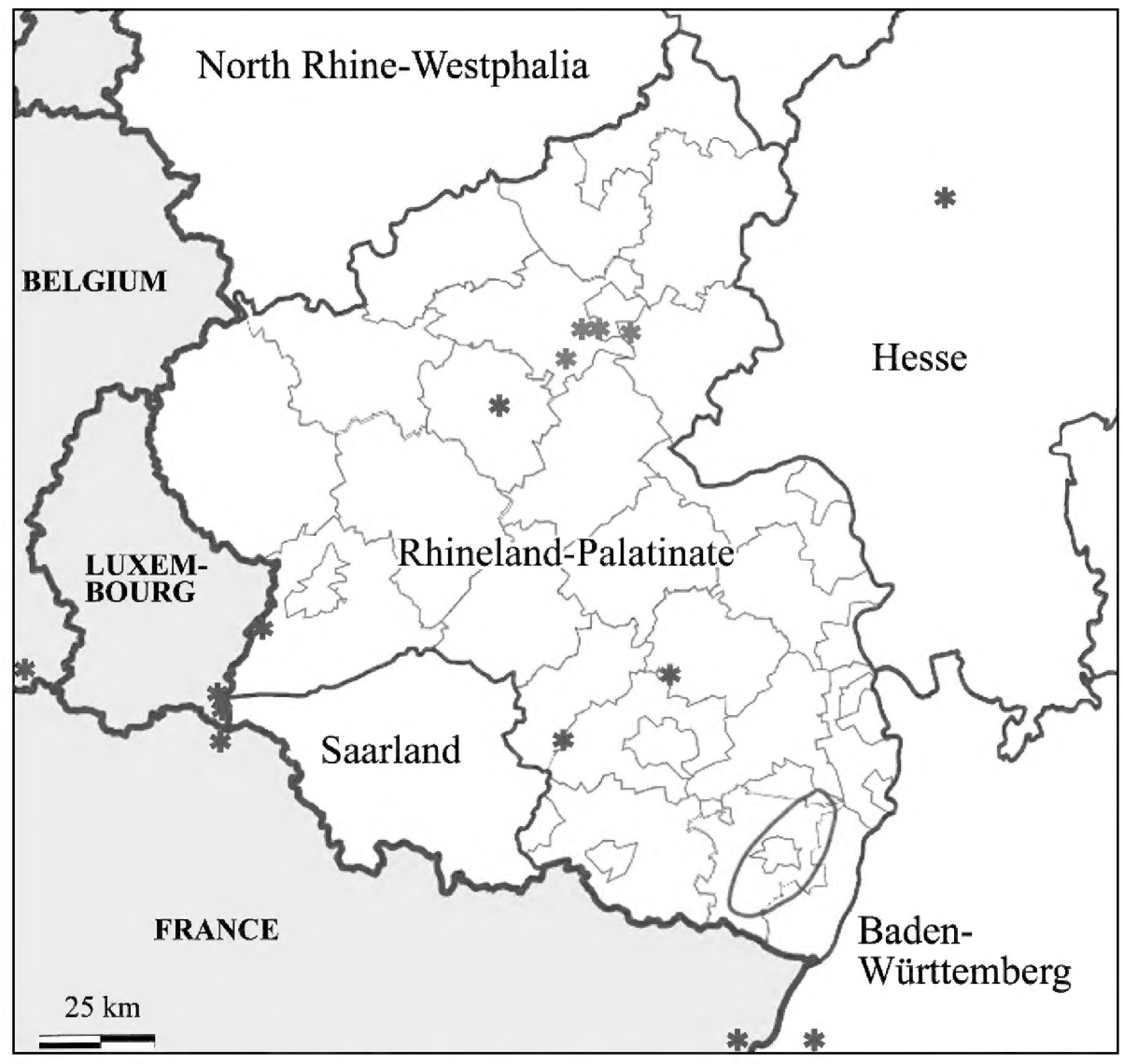


Figure 6. Map of positive and negative records of *Phlebotomus mascittii* in Rhineland-Palatinate, the neighboring federal states, and European countries. *Explanation of symbols*: Red = new positive sites (this study); Blue = negative sites (this study); Green = previously published positive sites. *Explanation of lines*: Green = area with previously published multiple positive records; Black = borders in descending width of European countries, federal states in Germany, districts in Rhineland-Palatinate.

50°19'N should be corrected as 50°10'N, and the given longitude of 7°15'E as 7°12'E, locating the trapping site about 5 km northeast of Cochem. Furthermore, T. Naucke (pers. comm.) told us that the Giessen specimen was most likely an artifact from an unauthorized release of some 2,000 living *Ph. mascittii*, which he had presented at a scientific meeting only about 800 m away and just six days prior to the collection date reported by Melaun et al. (2014).

Regarding trap types, we add two more types (color-LED and BG-Pro with CO_2) to previously mentioned traps, such as CDC light traps (Naucke and Schmitt 2004), BG-sentinel with CO_2 and UV light (Melaun et al. 2014), and sticky traps (Schaffner 2023). However, it has already been argued that some sand flies, such as *Ph. mascittii*, are "light-shy" and less attracted by carbon dioxide (Alten et al. 2015; Oerther et al. 2020), making most *Ph. mascittii* catches so far only random catches. We made a few trials using pieces of fruit as additional attractants (suggested by Poeppl et al. 2013) in 2022, but without success. It remains to be elucidated which stimuli are preferred by *Ph. mascittii*.

Considering ours and most of the previous as random catches implies that *Ph. mascittii* is more common and widespread. That in turn, combined with the suspected vector potential (Obwaller et al. 2016), would pose a risk—though manageable—of autochthonous *L. infantum* transmission. From a One Health perspective, including the military medicine context, intensified risk communication seems prudent.

References

Alten B, Özbel Y, Ergunay K, Kasap OE, Cull B, Antoniou M, Velo E, Prudhomme J, Molina R, Bañuls AL, Schaffner F, Hendrickx G, Van Bortel W, Medlock JM (2015) Sampling strategies for phlebotomine sand flies (Diptera, Psychodidae) in Europe. Bulletin of Entomological Research 105(6): 664–678. https://doi.org/10.1017/S0007485315000127

Depaquit J, Naucke TJ, Schmitt C, Ferté H, Léger N (2005) A molecular analysis of the subgenus *Transphlebotomus* Artemiev, 1984 (*Phlebotomus*, Diptera, Psychodidae) inferred from ND4 mtDNA with new northern records of *Phlebotomus mascittii* Grassi, 1908. Parasitology Research 95(2): 113–116. https://doi.org/10.1007/s00436-004-1254-x

Dantas-Torres F, Tarallo VD, Otranto D (2014) Morphological keys for the identification of Italian phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae). Parasites & Vectors 7: 479. https://doi.org/10.1186/s13071-014-0479-5

Kasbari M, Ravel C, Harold N, Pesson B, Schaffner F, Depaquit J (2012) Possibility of leishmaniasis transmission in Jura, France. Emerging Infectious Diseases 18(6): 1030. https://doi.org/10.3201/eid1806.120158

Maia C, Cardoso L (2015) Spread of *Leishmania infantum* in Europe with dog travelling. Veterinary Parasitology 213(1–2): 2–11. https://doi.org/10.1016/j.vetpar.2015.05.003

Maia C, Conceição C, Pereira A, Rocha R, Ortuño M, Muñoz C, Jumakanova Z, Pérez-Cutillas P, Özbel Y, Töz S, Baneth G, Monge-Maillo B, Gasimov E, Van der Stede Y, Torres G, Gossner CM, Berriatua E (2023) The estimated distribution of autochthonous leishmaniasis by *Leishmania infantum* in Europe in 2005–2020. PLOS Neglected Tropical Diseases 17(7): e0011497. https://doi.org/10.1371/journal.pntd.0011497

Melaun C, Krüger A, Werblow A, Klimpel S (2014) New record of the suspected leishmaniasis vector *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908 (Diptera, Psychodidae, Phlebotominae) – the northernmost phlebotomine sandfly occurrence in the Palearctic region. Parasitology Research 113(6): 2295–2301. https://doi.org/10.1007/s00436-014-3884-y

Naucke TJ, Pesson B (2000) Presence of *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908 (Diptera, Psychodidae) in Germany. Parasitology Research 86(4): 335–336. https://doi.org/10.1007/s004360050053

Naucke TJ, Schmidt C (2004) Is leishmaniasis becoming endemic in Germany? International Journal of Medical Microbiology 293(Suppl. 37): 179–181. https://doi.org/10.1016/S1433-1128(04)80036-6

Naucke TJ, Menn B, Massberg D, Lorentz S (2008) Sandflies and leishmaniasis in Germany. Parasitology Research 103(Suppl. 1): 65–68. https://doi.org/10.1007/s00436-008-1052-y

Naucke TJ, Lorentz S, Rauchenwald F, Aspöck H (2011) *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908, in Carinthia: first record of the occurrence of sandflies in Austria (Diptera, Psychodidae, Phlebotominae). Parasitology Research 109(4): 1161–1164. https://doi.org/10.1007/s00436-011-2361-0

Obwaller AG, Karakus M, Poeppl W, Töz S, Özbel Y, Aspöck H, Walochnik J (2016) Could *Phlebotomus mascittii* play a role as a natural vector for *Leishmania infantum*? New data. Parasites & Vectors 9: 458. https://doi.org/10.1186/s13071-016-1750-8

Oerther S, Jöst H, Heitmann A, Lühken R, Krüger A, Steinhausen I, Brinker C, Lorentz S, Marx M, Schmidt-Chanasit J, Naucke T, Becker N (2020) Phlebotomine sand flies in Southwest Germany: an update with records in new locations. Parasites & Vectors 13(1): 173. https://doi.org/10.1186/s13071-020-04058-6

Poeppl W, Obwaller AG, Weiler M, Burgmann H, Mooseder G, Lorentz S, Rauchenwald F, Aspöck H, Walochnik J, Naucke TJ (2013) Emergence of sandflies (Phlebotominae) in Austria, a Central European country. Parasitology Research 112(12): 4231–4237. https://doi.org/10.1007/s00436-013-3615-9

Prudhomme J, Depaquit J, Robert-Gangneux F (2024) Phlebotomine sand fly distribution and abundance in France: A systematic review. Parasite 31: 45. https://doi.org/10.1051/parasite/2024045

Ready PD (2013) Biology of phlebotomine sand flies as vectors of disease agents. Annual Review of Entomology 58: 227–250. https://doi.org/10.1146/annurev-ento-120811-153557

Risueño J, Bersihand S, Bender C, Cornen T, De Boer K, Ibáñez-Justicia A, Rey D, Rozier Y, Schneider A, Stroo A, Vanslembrouck A, Van Bortel W, Weigand A, Zambianchi D, Pérez Cutillas P, Oerther S, Braks M, Wint GRW, Berriatua E, Schaffner F (2024) A survey of Phlebotomine sand flies across their northern distribution range limit in Western Europe. Journal of the European Mosquito Control Association 42(2): 105–115. https://doi.org/10.52004/2054930x-20241008

Schaffner F (2023) Occurrence of *Phlebotomus mascittii* (Diptera: Psychodidae: Phlebotominae) in the Upper Rhine Valley of Alsace, France. Annales de la Société entomologique de France (N.S.) 59(4): 278–284. https://doi.org/10.1080/00379271.2023.2228741

Theodor O (1958) 9c. Psychodidae-Phlebotominae. In: Lindner E (Ed.) Die Fliegen der Palaearktischen Region. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 1–55.